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Multiple Forms of Cytochrome P450 in the Microsomal Monooxygenase System*

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Abstract. The microsomal monooxygenase system is characterized by its broad substrate specificity which includes endogenous substrates as well as lipophilic drugs and chemicals. From in vitro investigations it was known that the relative reactivities and the pattern of products varied greatly with species, sex, age, diet or pretreatment with drugs of the animal. The suggestion that this was possibly due to a variety of cytochrome P450 enzymes rather than a single monooxygenase was recently confirmed by the isolation of several cytochrome P450 species with different although overlapping substrate specificities. In view of the consequences of a genetic and environment-dependent pattern of monooxygenases for drug metabolism and drug-mediated toxicity the methods of a quantitative assessment of the various forms are discussed.

Key words: Cytochrome P450 – Multiple forms – Monooxygenase system – Inducers.

Introduction

Chemically used drugs may be considered as only a relatively small number of organic chemicals to which living organisms in our biosphere are increasingly exposed. All of these compounds, also called "xenobiotics", do not enter into the normal metabolic routes of the organism and hence must be excreted. This occurs readily with water soluble xenobiotics, but not with lipophilic compounds. These must first be converted to more polar derivatives in order to change their partition coefficient in favor of the aqueous compartment. Living organisms have developed

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enzymatic oxidation systems, so-called monooxygenases, which are using molecular oxygen for the introduction of an oxygen atom into organic molecules (Ullrich, 1972). In both man and the higher organisms, the monooxygenase activity is found mainly in the endoplasmic reticulum of liver. However, the small intestine, lung, skin, kidney or lymphocytes also contain these enzymes. The same enzymatic complex is involved in the metabolism of several endogenous compounds such as steroid hormones and fatty acids. If NADPH and oxygen are provided as cofactors, the monooxygenation of drugs or lipophilic compounds can be studied in vitro on the microsomal fraction of a cell homogenate.

The most striking property of the microsomal monooxygenase system is its lack of specificity which permits the attack of practically all lipophilic compounds. Therefore, the question arose of whether a single cytochrome P450 could exert this unspecificity or whether a multiplicity of monooxygenase was required.

This has been a controversial question for a number of years and many scientists in the field favored the idea of only one monooxygenase system with a corresponding broad substrate specificity. However, a variety of observations were not consistent with the existence of only one enzyme and the recent advances in the solubilization of the microsomal monooxygenase system with its resolution into a flavoprotein and several cytochrome P450 enzymes leave no doubt about the heterogeneity of the drug monooxygenase system. Since this is of fundamental importance in the phenomenon of drug toxicity the various aspects of the different forms of cytochrome P450 will be evaluated in more detail.

Evidence for the Heterogeneity of the Microsomal Drug Monooxygenase System

Assuming that a given enzyme can react with more than one substrate, one can predict that the corresponding specific activities will be different but should remain in constant proportions. On the basis of this assumption one could have previously argued against a single monooxygenase for xenobiotics, since liver microsomes were found to exhibit large differences in reactivity towards various substrates depending on the animal species, sex, age, diet or pretreatment with drugs (Gillette, 1966, 1971; Kato, 1977; Conney, 1967; El Delfrawy et al., 1974; Carpenter and Howard, 1974). That enzymes from different species can vary may be readily understandable, however sex differences or different reactivities after certain diets or drug exposure are hardly compatible with a single enzyme. In particular phenobarbital pretreatment versus that of polycyclic hydrocarbons was followed by large differences in substrate specificities. Phenobarbital (Pb) often increases the specific activities of the liver microsomal fraction towards aliphatic compounds, whereas benzo(a)pyrene (Bp) exerts a strong stimulatory effect mainly on the hydroxylation of aromatic substrates (Table 1).

In addition to the specific activities and hence the substrate specificities the two different pretreatments also altered the patterns of products. As a consequence of a nonstereospecific binding of substrates to the active site of an enzyme one can expect more than one product to be formed if the substrate molecule can be attacked at several positions. Again, if we were dealing with one monooxygenase this pattern

Table 1. Specific activities for various substrates in male rat liver microsomes after pretreatment with phenobarbital and benzo(a)pyrene

Substrate	Controls	Spec. activity[a]/nmol × mg protein^{-1} × min^{-1}		Reference
		Phenobarbital	Benzo(a)pyrene	
Cyclohexane	4.2	22.2	3.1	Frommer et al. (1974)
n-Hexane	1.8	8.5	1.7	Ullrich et al. (1969)
7-Ethoxycoumarin	0.2	2.2	5.4	Ullrich et al. (1975)
Acetanilide	0.8	1.3	4.5	Ullrich et al. (1969)
7-Ethoxyresorufin	0.15	0.2	18.5	Kremers, unpublished

[a] Total products were determined

of products should be constant. However, numerous examples indicate that the relative amounts of products vary to a large extent when the above mentioned parameters such as species, diet, sex, age or pretreatment were modified (Kato, 1977; Carpenter and Howard, 1974; Tredger et al., 1976; Goujon et al., 1972). The most striking differences were observed after pretreatment with phenobarbital and polycyclic hydrocarbons. Table 2 contains a list of substrates which have been investigated in rat liver microsomes with regard to their product pattern.

The differences observed in the products not only confirm qualitative differences between the monooxygenase system induced by phenobarbital and benzpyrene, but also allow a chemical differentiation and generalization from the profile of products. One finds that Pb-microsomes exhibit a preference for the ω-1 position when alkanes are used as substrates (Frommer et al., 1970a, b; Frommer et al., 1974). The methylene groups in ω-2 or ω-3 position are chemically essentially equal to each other and should also be attacked unless steric hindrance restricts the hydroxylation at these positions. Chemical models in which the active oxygen species is not bound to an enzyme but free in solution do not discriminate between the ω-1, ω-2 or ω-3 position indicating that steric effects indeed are responsible for the preferential hydroxylation in the ω-1 position by Pb-microsomes (Frommer and Ullrich, 1971; Ullrich et al., 1972). However, by comparison with the hydroxylation products obtained in Bp-microsomes one finds much less stereoselectivity with regard to the ω-1, ω-2 and ω-3 position (Frommer et al., 1974). The rather high ortho-hydroxylation of diphenyl points to the same effect since an electrophilic active oxygen would tend to attack the ortho-position as well as the para-position, but the ortho-position is always sterically hindered. An even more sophisticated example of the controversial influence of reactivity and stereoselectivity is seen in the monooxygenation of 7-methoxycoumarin (Nastainczyk et al., in preparation). The O-dealkylation product umbelliferone is the main metabolite in Pb-microsomes whereas the ring-hydroxylation product predominates in Bp-microsomes. For an electrophilic oxenoid active oxygen an attack of the aromatic ring by addition to the π-electron system would be the pathway of lowest activation energy as can be shown by model hydroxylations (Lichtenberger et al., 1976), but obviously steric inhibition at the ring is favoring the attack of the O-methyl group in Pb-microsomes. Bp-microsomes preferentially at-

Table 2. Effect of pretreatment by polycyclic hydrocarbons and phenobarbital on the product pattern of microsomal monooxygenations in rats

Substrate	Products	% of total metabolites		Reference
		Pb	Bp[a] or 3-MC[b]	
n-Hexane	n-Hexanol-1	6	12[a]	Frommer et al.
	n-Hexanol-2	78	47	(1974)
	n-Hexanol-3	16	41	
Testosterone	6β-OH-Testosterone	37	26[b]	Conney et al.
	7α-OH-Testosterone	37	73	(1969)
	16α-OH-Testosterone	26	1	
Biphenyl	2-OH-Biphenyl	5	38	Burke and Mayer
	2-OH-Biphenyl	95	62	(1975)
7-Methoxycoumarin	Umbelliferone	81	28	Nastainczyk et al.
	6-OH-7-methoxycoumarin	19	72	(unpublished)

tack the ring, supporting the smaller influence of stereoselectivity in these microsomes. One could argue that here the O-alkyl group is sterically shielded from an attack by the active oxygen, however 7-ethoxycoumarin is mainly O-dealkylated because of the higher reactivity of the secondary CH-bonds adjacent to the ether oxygen atom, which proves that steric hindrance plays no major role in the monooxygenation by the monooxygenase system in Bp-microsomes.

The different nature of the monooxygenase systems after pretreatment by phenobarbital and benzpyrene is also reflected by a different pH-optimum and a lower K_m-value for 7-ethoxycoumarin in Bp-microsomes (Ullrich and Weber, 1972). In general it seems that the cytochrome P450 induced by polycyclic hydrocarbons has a very high affinity for aromatic substrates in contrast to the Pb-induced form.

When the nature of the microsomal monooxygenase system was investigated more closely even spectral differences between the cytochrome P450 systems induced by phenobarbital and benzpyrene or 3-methylcholanthrene were obtained. In the latter case the Soret band of the carbon monoxide complex was shifted to 448 nm compared to 450 nm and ethyl isocyanide produced two different bands with different relative intensities and a different pH-dependence (Bohn et al., 1971; Hildebrandt et al., 1968; Haugen et al., 1976; Comai and Gaylor, 1973).

The Soret absorption of the oxidized cytochrome was centered around 418 nm in livers from phenobarbital pretreated animals whereas it was found around 390 nm after benzpyrene induction. According to EPR-measurements this corresponded to low and high spin states of the cytochrome, respectively.

Lipophilic compounds readily convert the low spin cytochrome P450 of Pb-microsomes to the high spin enzyme substrate complex, but since the Bp-induced cytochrome is already mainly in the high-spin state only minor spectral changes are seen in these microsomes.

Related to a possibly greater thermodynamic stability of the high spin form in Bp-microsomes may be the observation that organic ligands to cytochrome P450,

like metyrapone (Hildebrandt et al., 1969), sulfides (Nastainczyk et al., 1975), thiols (Nastainczyk et al., 1976) or phosphines (Mansuy et al., 1974) show a much better binding to cytochrome P450 of Pb-microsomes than to the cytochrome of Bp-microsomes. This may be the reason why metyrapone proved to be a good inhibitor for monooxygenations catalyzed by Pb-microsomes but not by Bp-microsomes (Frommer et al., 1974).

Qualitative differences in the monooxygenase systems were not only observed after the two different pretreatments but also between microsomes of Pb-induced animals and those of controls (Comai and Gaylor, 1973; Mailman et al., 1975). These results suggest that a family of cytochrome P450 species must exist with different substrate specificities and different product patterns (Mailman et al., 1975; Gustafsson and Ingelman-Sundberg, 1976). This concept was unequivocally established by the isolation of cytochrome P450 from animals of controls and after pretreatment with phenobarbital and benzpyrene (Haugen and Coon, 1976). The electrophoretically homogeneous cytochrome P450 enzymes showed essentially the same substrate and product specificities in reconstituted systems as in the corresponding microsomal fractions.

Interestingly, however, disc electrophoresis of total microsomes revealed several cytochrome P450 species of different molecular weights even in Pb- and Bp-pretreated animals. Compared to controls, pretreatment seemed to increase mainly one or two forms whereas the others seemed to decrease. From a hybrid strain of mice pretreated with phenobarbital four forms of cytochrome P450 have been isolated which showed different relative activities and product distributions towards testosterone, benzphetamine, ethylmorphine and 7-ethoxycoumarin (Haugen et al., 1976). It is therefore quite certain, that every organism is able to synthesize a variety of cytochrome P450 species with different but often overlapping substrate specificities and different product patterns. All variations with species, sex, diet or environment could then be a consequence of differences in the relative amounts of the various cytochrome P450 forms.

A Hypothesis Concerning the Heterogeneity of Microsomal Monooxygenases

The diversity of cytochrome P450 enzymes is analogous to that of the esterases (Krisch, 1971) but probably not more than 7 or 10 species of cytochrome P450 may be present in microsomes (Gunsalus et al., 1975). In a strict sense these forms cannot be regarded as multiple forms or isoenzymes since they do not possess the same substrate specificity. The evolutionary advantage of more than one monooxygenase becomes evident if one considers the efficiency of the elimation of drugs and foreign lipophilic compounds from the body under in vivo conditions. A single, completely unspecific monooxygenase must necessarily exhibit low affinities to substrates since unspecificity and high affinity of enzymes are mutually exclusive. On the other hand environmental contaminants like benzpyrene, DDT or foreign lipophilic food constituents are usually present in only small amounts, so that high affinities are required to effectively remove these compounds from the body. Thus a limited number of more selective enzymes can deal better with the required affinity.

A second advantage of multiple forms becomes obvious if one looks at the biosynthetic regulation of the monooxygenase activity. The effect of pretreatment of an organism corresponds to an induction which increases the steady state concentration of a microsomal cytochrome P450 by either increasing its synthesis and/or decreasing its breakdown. Instead of inducing the formation of large amounts of a less specific and less efficient enzyme it is more economic to produce smaller amounts of a more specific enzyme. This would require, however, that the inducer enhances the steady state concentration of those cytochrome P450 enzymes which catalyze the monooxygenation of the inducer. This seems to be true for the polycylic hydrocarbons and also for the barbiturates but remains to be established for other groups of inducers. There are recent reports which suggest that other inducers like polyhalogenated aromatic compounds (Stonard and Greig, 1976), insecticide synergists (Birnbaum et al., 1976; Kramer and Colby, 1976) or steroid analogues can cause patterns of cytochrome P450 forms different from that of phenobarbital and benzpyrene. In contrast, the absence of xenobiotics as inducers would be followed by a decrease in the steady state levels of cytochromes P450 as can be observed in mouse small intestine if a synthetic diet is provided for several days and any organic bedding material is removed (Boobis et al., 1976). The half-life times of the various cytochrome P450 forms are different (Haugen et al., 1976) but are probably in the range of 10—20 h so that a sufficiently fast regulation of their steady-states can be maintained.

According to this hypothesis the quality and quantity of the inducer would be important for the cytochrome P450 pattern, but also other factors are involved. Genetic differences between various strains of mice have been described which point to the role of special receptors for the inducers (Haugen et al., 1976). In case of a lacking or non-functional receptor the induction could be abolished. But also environmental factors, such as protein deficiency, diseases or the hormonal status may vary protein synthesis or breakdown and thereby modify the induction process. Many of these factors should be reinvestigated in terms of their influence on the cytochrome P450 pattern.

Determination of the Heterogeneity of the Microsomal Monooxygenase System

Since the cytochrome P450 pattern of the microsomal monooxygenase system is a major determinant of the quantity and quality of drug metabolism in man and other species, it may be appropriate to mention the techniques available for the characterization of the single forms of cytochrome P450. The only biochemical approach to this problem is the isolation of each cytochrome from a given species followed by the combination with NADPH-cytochrome P450 reductase in order to study the substrate and product specificity as well as the kinetic parameters of the reconstituted monooxygenases. For routine studies concerning the influence of various parameters on the relative distribution of the different cytochrome P450 forms less time consuming assays could be introduced. If the cross-reactivity is not too high specific antibodies could be a first possibility (Thomas et al., 1976). As a simplest procedure it may be sufficient to separate all forms electrophoretically and measure their rela-

Table 3. Effect of inhibitors on the liver microsomal O-dealkylation of 7-ethoxycoumarin after various pretreatments of rats. The values represent means (± SD) of 6–8 rats (Ullrich et al., 1975)

Pretreatment		None (controls)		Phenobarbital (♂ + ♀)[a]	Benzpyrene (♂ + ♀)[a]
Sex		♂	♀		
Specific activity nMol/mg prot. min		0.2 ± 0.1	0.05 ± 0.01	2.2 ± 0.3	5.4 ± 1
% Inhibition	2×10^{-6}	30 ± 2	0 ± 1	29 ± 2	0 ± 1
by	4×10^{-6}	45 ± 6	1 ± 1	39 ± 3	0 ± 1
Metyrapone	8×10^{-6}	50 ± 7	2 ± 1	60 ± 5	0 ± 1
	2×10^{-5}	52 ± 5	5 ± 2	72 ± 6.5	0 ± 1
% Inhibition	$2{,}5 \times 10^{-6}$	− 3 ± 1	0 ± 0.5	0 ± 0.5	12 ± 3
by	5×10^{-6}	− 3 ± 1	0 ± 1	2 ± 1	42 ± 5
7,8-Benzoflavone	1×10^{-5}	3 ± 2	5 ± 2	3 ± 1	79 ± 4
	2×10^{-5}	5 ± 2.5	12 ± 3	3 ± 1	90 ± 3
% Inhibition	1×10^{-3}	4 ± 1	14 ± 3	0 ± 1	0 ± 1
by	2×10^{-3}	6 ± 1	24 ± 4	0 ± 1	1 ± 0.5
Tetrahydrofuran	5×10^{-3}	10 ± 2	29 ± 7	3 ± 1	2 ± 1
	1×10^{-2}	15 ± 3	48 ± 10	4 ± 1	2 ± 1

[a] No significant differences observed

Table 4. O-dealkylation of 7-ethoxycoumarin in human liver biopsy samples (Ullrich, unpublished)

Patient	Sex	Age	Specific activity[a]	% Inhibition		
				Metyr.[b]	THF[c]	7,8-Bfl[d]
S., T.	m.	32	0.10	27	52	16
L., E.	f.	47	0.12	37	38	10
A., E.	m.	–	0.12	33	42	32
Z., A.	f.	55	0.00	–	–	–
B., E.	f.	63	0.10	22	25	33
R., R.	f.	25	0.02	0	100	0
M., M.	m.	21	0.15	27	52	31
R., G.	m.	30	0.35	16	41	0
B., H.	m.	38	0.04	28	70	46
S., K.	f.	70	0.14	33	34	84
C., P.	m.	47	0.30	30	28	22
C., K.	m.	57	–	48	52	27
Z., J.	m.	20	0.13	20	35	95
F., K.	m.	29	0.04	10	67	17
S., K.	m.	41	0.05	22	44	19
F., H.	m.	40	0.04	50	80	33
K., A.	m.	63	0.02	0	10	72
B., J.	m.	–	0.03	39	50	0
W., A.	m.	66	0.02	5	25	70

[a] nMol umbelliferone × mg^{-1} protein of 500 × *g* supernatant × min^{-1}
[b] 10^{-5} M metyrapone (2-methyl-1,2 bis-(3-pyridyl)-1-propanone)
[c] 10^{-3} M tetrahydrofuran
[d] 10^{-5} M 7,8-benzoflavone

tive intensities. It may also be possible to obtain selective inhibitors for each form as was already shown for rat liver microsomes after three different pretreatments (Table 3).

In an attempt to apply these inhibitors also to man we have determined the inhibition pattern with these three inhibitors in human biopsy samples. About 10 mg of biopsy material was homogenized and the activity for 7-ethoxycoumarin dealkylation was measured in the 300 × *g* supernatant after addition of the three inhibitors. The results showed a variation in the total O-dealkylation activity and also considerable differences in the relative percentage of inhibition.

Since human cytochrome P450 forms have not yet been isolated, no quantitative data on the multiplicity of the cytochrome P450 forms in human liver are known but the inhibition pattern indicates that such a multiplicity is likely to exist also in man. This brings us to the question about the significance of qualitative and quantitative differences in the monooxygenase system for drug metabolism and drug toxicity.

Consequences of the Existence of Different Cytochrome P450 Forms on Drug Metabolism and Drug Toxicity

For a long time it has been understood that the activity of the monooxygenase system in the liver and other organs is correlated with the excretion of lipophilic drugs and chemicals. Hence the duration of action of a drug or the toxicity of a chemical is also a function of this monooxygenase activity (Conney, 1967). It should be kept in mind, however, that in principle it is not the content of cytochrome P450 which correlates with the rate of metabolism of a given drug but only the monooxygenase activity measured in vitro against the same drug. Even then, one must account for the in vivo concentrations of this drug since the affinities of the various cytochrome P450 forms may differ by about two orders of magnitude. Practically, however, a rough correlation between in vitro and in vivo data can be obtained. Exceptions may occur in the diseased state and after pretreatment by various drugs (Kato, 1977).

The existence of many cytochrome P450 forms may be very important for the studies on the mutual interaction of two drugs. If the binding of the two compounds occurs at the same form of cytochrome P450, a maximal interaction and inhibition in monooxygenation is seen, whereas essentially no effect may be observed if in admittedly rare cases both drugs bind to different portions of microsomal cytochrome P450.

It must also be kept in mind that several endogenous compounds are deactivated and metabolized by the cytochrome P450 dependent microsomal monooxygenases. The interference of drugs and endogenous compounds, namely steroids, at the cytochrome P450 level, may be of two types:

i) a competition of drug and steroid molecules for the same cytochrome P450 molecule and subsequent competitive inhibition of the monooxygenase activity;

ii) an induction of the steroid hydroxylation activity by the drug leading to a decreased or increased blood level of the hormone and hence to more or less important hormonal disorders. Some pharmacological implications of microsomal enzyme induction have been reviewed by Conney (1967).

All consequences of the multiplicity of cytochrome P450 mentioned so far may affect the rate of metabolism of a lipophilic compound and therefore are not very spectacular. This changes if we consider a drug or a chemical which can be metabolized to a toxic product. Examples of harmful metabolites are N-hydroxy compounds or epoxides (Oesch, 1976) which may bind to macromolecules of the cell and even can generate cancerous cells (Glatt, 1975). Since the product pattern of the various cytochrome P450 forms may differ largely it becomes now very important to determine whether by the same rate of metabolism one form of cytochrome P450 leads to the formation of reactive metabolites or to the formation of stable alcohols or phenols. Examples of toxification and inactivation pathways in dependence on the cytochrome P450 forms are known today for the carcinogen benzpyrene (Jernström et al., 1976) and many more examples may be found in the future.

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References

Birnbaum, L. S., Baird, M. B., Massie, H. R.: Pregnenolone-16α-carbonitrile inducible cytochrome P450 in rat liver. Res. Commun. Chem. Path. Pharmacol. **15,** 553–562 (1976)

Bohn, W., Ullrich, V., Staudinger, Hj.: Species of cytochrome P450 in rat liver microsomes with different stereoselectivity for the binding and monooxygenation of (+) and (–) methylphenobarbital. Naunyn-Schmiedebergs Arch. Pharmak. **270,** 41–45 (1971)

Boobis, A. R., Nebert, D. W., Felton, J. S.: Comparaison of β-naphtoflavone and 3-methylcholanthrene as inducers of hepatic cytochrome(s) P448 and aryl hydrocarbon hydroxylase activity. Molec. Pharmacol. **13,** 259–268 (1976)

Burke, M. D., Mayer, R. T.: Inherent specificities of purified cytochrome P450 and P448 toward biphenyl hydroxylation and ethoxyresorufin deethylation. Drug Metab. Disp. **3,** 245–253 (1975)

Carpenter, M. C., Howard, Ch. N.: Vitamin E, steroids and liver microsomal hydroxylation. Amer. J. clin. Nutr. **27,** 966–979 (1974)

Comai, K., Gaylor, J. L.: Existence and separation of three forms of cytochrome P450 from rat liver microsomes. J. biol. Chem. **248,** 4947–4955 (1973)

Conney, A. H.: Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. **19,** 317–366 (1967)

Conney, A. H., Levin, W., Jacobsson, M., Kuntzman, R.: Specificity in the regulation of the 6β, 7α and 16α-hydroxylation of testosterone by rat liver microsomes. In: Microsomes and drug oxidations (J. R. Gillette, ed.), pp. 279–301. New York: Academic Press 1969

El Defrawy El Masry, S., Cohen, G. M., Mannering, G. D.: Sex dependent differences in drug metabolism in the rat. Drug Metab. Disp. **2,** 267–300 (1974)

Frommer, U., Ullrich, V.: Hydroxylation of aliphatic compounds by liver microsomes (III): Model hydroxylation reactions. Z. Naturforsch. **26,** 322–327 (1971)

Frommer, U., Ullrich, V., Staudinger, Hj.: Hydroxylation of aliphatic compounds by liver microsomes (I). Hoppe-Seylers Z. physiol. Chem. **351,** 903–912 (1970a)

Frommer, U., Ullrich, V., Staudinger, Hj.: Hydroxylation of aliphatic compounds by liver microsomes (II). Hoppe-Seylers Z. physiol. Chem. **351,** 913–918 (1970b)

Frommer, U., Ullrich, V., Orrenius, S.: Influence of inducers and inhibitors on the hydroxylation pattern of n-hexane in rat liver microsomes. FEBS Lett. **41,** 14–16 (1974)

Gillette, J. R.: Biochemistry of drug oxidation and reduction by enzymes in hepatic endoplasmic reticulum. Advanc. Pharmacol. **4,** 219–261 (1966)

Gillette, J. R.: Effect of various inducers on electron transport system associated with drug metabolism by liver microsomes. Metabolism **20,** 215–227 (1971)

Glatt, H. R., Oesch, F., Frigerio, A., Garattini, S.: Epoxides metabolically produced from some known carcinogens and from some clinically used drugs. Int. J. Cancer **16,** 787–797 (1975)

Goujon, F. M., Nebert, D. W., Gielen, J. E.: Genetic expression of aryl hydrocarbon hydroxylase induction. Molec. Pharmacol. **8,** 667–680 (1972)

Gunsalus, I. C., Pederson, T. C., Sligar, S. G.: Oxygenase-catalysed biological hydroxylations. Ann. Rev. Biochem. **44,** 377–407 (1975)

Gustafsson, J. A., Ingelman-Sundberg, M.: Multiple forms of cytochrome P450 in rat liver microsomes. Europ. J. Biochem. **64,** 35–43 (1976)

Haugen, D. A., Coon, M. J.: Properties of electrophoretically homogeneous phenobarbital inducible and β-naphthoflavone inducible forms of liver microsomal cytochrome P450. J. biol. Chem. **251,** 7929–7939 (1976)

Haugen, D. A., Coon, M. J., Nebert, D. W.: Induction of multiple forms of mouse liver cytochrome P450. J. biol. Chem. **251,** 1817–1827 (1976)

Hildebrandt, A. G., Remmer, H., Estabrook, R. W.: Cytochrome P450 of liver microsomes, one pigment or many? Biochem. biophys. Res. Commun. **30,** 607–612 (1968)

Hildebrandt, A. G., Leibman, R. C., Estabrook, R. W.: Metyrapone interaction with hepatic microsomal cytochrome P450 from rats treated with phenobarbital. Biochem. biophys. Res. Commun. **37,** 477–485 (1969)

Jernström, B., Vadi, H., Orrenius, S.: Formation in isolated rat liver microsomes and nuclei of benzo(a)pyrene metabolites that bind to DNA. Cancer Res. **36,** 4107–4113 (1976)

Kato, R.: Drug metabolism under pathological and abnormal physiological states in animal and man. Xenobiotica **7,** 25–92 (1977)

Kramer, R. E., Colby, H. D.: Feminization of hepatic steroid and drug metabolizing enzymes by growth hormone in male rats. J. Endocr. **71,** 449–450 (1976)

Krisch, K.: Carboxylic ester hydrolases. In: The enzymes (P. D. Boyer, ed.), Vol. 5, pp. 43–69. New York: Academic Press 1971

Lichtenberger, F., Nastainczyk, W., Ullrich, V.: Cytochrome P450 as an oxene transferase. Biochem. biophys. Res. Commun. **70,** 939–946 (1976)

Mailman, R. B., Tate, L. G., Muse, K. E., Coons, L. B., Hodgson, E.: The occurence of multiple forms of cytochrome P450 in hepatic microsomes from untreated rats and mice. Chem. Biol. Interactions **10,** 215–228 (1975)

Mansuy, D., Duppel, W., Ruf, H. H., Ullrich, V.: Phosphines as ligands to microsomal cytochrome P450. Hoppe-Seylers Z. physiol. Chem. **355,** 1341–1349 (1974)

Nastainczyk, W., Ruf, H. H., Ullrich, V.: Ligand binding of organic sulfides to microsomal cytochrome P450. Europ. J. Biochem. **60,** 615–620 (1975)

Nastainczyk, W., Ruf, H. H., Ullrich, V.: Binding of thiols to microsomal cytochrome P450. Chem. Biol. Interactions **14,** 251–263 (1976)

Oesch, F.: Differential control of rat microsomal "aryl hydrocarbon" monooxygenase and epoxide hydratase. J. biol. Chem. **251,** 79–87 (1976)

Stonard, M. D., Greig, J. B.: Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. Chem. Biol. Interactions **15,** 365–379 (1976)

Thomas, P. E., Lu, P. Y. H., Ryan, D., West, S. B., Kawalek, J., Levin, W.: Immunochemical evidence for six forms of rat liver cytochrome P450 obtained using antibodies against purified rat liver cytochrome P450 and P448. Molec. Pharmacol. **12,** 746–758 (1976)

Tredger, J. M., Chakraborty, J., Parke, D. V.: Effect of natural and synthetic glucocorticoids on rat hepatic microsomal drug metabolism. J. Steroid Biochem. **7,** 351–356 (1976)

Ullrich, V.: Enzymatic hydroxylation with molecular oxygen. Angew. Chem. int. edit. **11,** 701–712 (1972)

Ullrich, V., Weber, P.: The O-dealkylation of 7-ethoxycoumarin by liver microsomes. Hoppe-Seylers Z. physiol. Chem. **353,** 1171–1177 (1972)

Ullrich, V., Schädelin, J., Staudinger, Hj.: Hydroxylation and substrate binding of cyclohexane by liver microsomes. FEBS symposium **16,** 261–265 (1969)

Ullrich, V., Ruf, H. H., Mimoun, H.: Model systems for mono-oxygenases in biological hydroxylation mechanisms (B. S. Boyd, R. M. S. Smelle, eds.). New York: Acad. Press 1972

Ullrich, V., Weber, P., Wollenberg, P.: Tetrahydrofurane – an inhibitor for ethanol induced liver microsomal cytochrome P450. Biochem. biophys. Res. Commun. **64,** 808–813 (1975)

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